

# THE MODIFIABILITY OF THE DIURNAL PIGMENTARY RHYTHM IN ISOPODS

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It has been reported by several observers, among them Kleinholz (1937), that the diurnal variation in the pigment cell dispersion of isopods persists for some days after the animals have been kept in continuous darkness under laboratory conditions. Many other examples of the persistence of diurnal rhythms of one kind or another are given in the reviews of Jores (1935) and of Welsh (1938), the latter also describing some unsuccessful attempts to replace the 24-hour rhythm of animals by a 16-hour one through subjecting them to the influence of "artificial days" (8 hours of light and 8 of darkness). As recently reported (1939), our own experiments on the modification of the diurnal body temperature cycle in man by following a 21- or a 28-hour regime of living were quite successful in some individuals and not in others, indicating a strong individual variation in susceptibility to such modification. Realizing that greater numbers of individuals had to be studied in order to bring out decided group tendencies, it was determined to perform an experiment on animals, and the Bermudan isopod, *Ligia baudiniana*, already studied by Kleinholz, was chosen for the test.

These isopods can be seen in great numbers on the rocky shores near the Bermuda Biological Station and are easily captured. The problem of keeping them alive for more than 2-3 days under laboratory conditions was solved, after some trials, by placing them in individual 150-cc. wide-mouthed bottles, containing a small amount of sea-water and tilted slightly, so that the isopod could remain on either the dry or the wet portion of the bottom of the bottle. A small amount of food in the form of vegetative matter scraped off the wet rocks where the isopods abounded was put into the bottles and replenished as needed. Pieces of cheesecloth were stretched across the mouths of the bottles, permitting air to circulate but preventing the escape of the isopods. Under these conditions some animals remained alive and active for one to two weeks, others for much longer periods.

Only those specimens that were quite dark when captured in the daytime and that showed themselves to be very light during the night following were retained for further observation. They were usually

divided at random into two groups, which were placed in separate dark-rooms. In one of these rooms darkness prevailed all the time, while in the other light and darkness were alternated automatically by a time clock on an 18-hour cycle of 10 hours of light and 8 hours of darkness. The animals were always observed under uniform conditions of illumination, and a simple system of recording the degree of pigment dispersion was adopted: isopods were classed as definitely dark (score of 2), intermediate (score of 1) or distinctly light (score of 0). The observations were dictated to an assistant, and duplicate observations usually yielded the same scores. The group scores were obtained by adding the individual ones. Thus, a group of 20 isopods might have a pigmentation score as high as 40 and as low as zero. Observations were made 4-6 times per 24 hours, fairly evenly distributed.

The composite data obtained on six groups of isopods, from 16 to 30 to a group, are plotted in curve *A*. It will be seen that after a flattening out of the 24-hour pigmentary activity curve the animals subjected to an 18-hour cycle of light and darkness swung into a distinctly 18-hour pigmentary rhythm. The six separate group curves upon which *A* was based were practically superimposable and were all characterized by three features: (1) an initial period of 24 to 48 hours duration in which the pigmentary rhythm was still definitely diurnal; (2) a transition period, setting in earlier in groups that were started with a period of darkness beginning at noon and later in groups that were first subjected to darkness at dusk (6 P.M.), but in all groups occurring when the repetitive 8-hour shift of darkness commenced at midnight; and (3) a period of 18-hour pigmentary rhythms which may be termed paradoxical in that the greatest pigment dispersion occurred during the artificial darkness and not during the hours of light, as it takes place in nature.

Four groups of isopods, from 12 to 18 per group, were kept in continuous darkness, and these animals preserved their 24-hour cycle of pigmentary activity, as shown in composite curve *B*. They were dark during the daytime hours and pale at night, confirming the observations of Kleinholz. One group was first maintained in continuous darkness, behaving as stated above, and then subjected to the artificial 18-hour cycle. These isopods showed the same three periods of adjustment to the new routine, as did the animals that were under the influence of the 18-hour alternation of light and darkness from the time they were captured.

One group of 20 isopods was maintained under a reversed routine of darkroom illumination (darkness in the daytime and light at night), but it preserved the normal diurnal rhythm of pigmentary activity.

That means, of course, that these isopods were dark during the periods of artificial darkness (daytime) and pale when illuminated (night), resembling both the pigmentary behavior of the animals kept under continuous darkness and the paradoxical pigmentary alternations of the animals that became adjusted to the 18-hour routine.

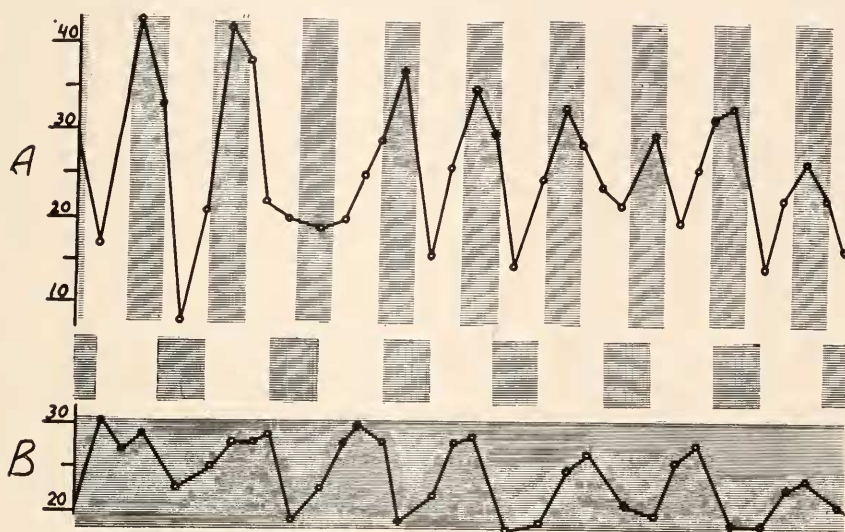


FIG. 1. Periodic variation in pigment dispersion, shown by groups of isopods, kept in the laboratory under artificial conditions of illumination and darkness. Curve *A* is based on the averages of six curves, obtained on groups comprising 30, 30, 20, 20, 16, and 20 animals, respectively. Maximal average pigment dispersion would be plotted as 46, maximum concentration, as zero. The general downward trend of the curve is due to the death of some animals, the total number decreasing from 136 to 104. The alternating areas of gray and white correspond to successive periods of 8 hours of darkness and 10 of light. Curve *B* is based on the averages of four curves, obtained on groups of 13, 12, 18, and 17 animals, respectively. Maximum pigment dispersion is plotted as 30, maximum concentration as zero. As in curve *A*, the downward trend of this curve is due to the death of individual isopods, whose total number decreased from 60 to 50. The gray background of curve *B* indicates the continuous darkness in which these groups were maintained. The alternating areas of gray and white between the two curves correspond to the natural 24-hour succession of night (7 P.M. to 5 A.M.) and day.

Although the occurrence of group pigmentary rhythms is unmistakable, the same cannot be said of individual animals. The groups studied were twice-selected isopods. It will be recalled that only those animals that were quite dark at the time of collection (daytime) were captured, in the first place. From 10 to 25 per cent of the isopods seen on the shore rocks were distinctly pale in the daytime. However, not all the animals that were dark when collected became light-colored

during the ensuing night in the laboratory. At least 10–15 per cent remained coal-black at night, and these were also rejected. Thus, the isopods retained for further observation had definitely changed from dark in the daytime to light at night. In the continued manifestation of a pigmentary rhythm the animals in captivity showed marked individual variation. Some isopods closely conformed to the group curves; others were either dark or light most of the time; still others were dark when the group was light and vice versa; finally, there were those whose pigmentary changes followed the group rhythm for one or another portion of the period of observation. As under the natural conditions of alternating day and night, so under the two artificial regimes to which the several groups of isopods were subjected, there were many individual deviations from the general group patterns of rhythmical dispersion and concentration of pigment.

#### SUMMARY

Although groups of Bermudan isopods, *Ligia baudiniana*, preserve their natural diurnal pigmentary rhythm when kept in total darkness for several days, they rapidly acquire an artificial 18-hour pigmentary rhythm, if exposed to alternating periods of 10 hours of light and 8 of darkness. The new rhythm is paradoxical, pigment cell dispersion occurring during darkness, instead of during the hours of illumination.

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